Report on the techniques for the analysis of chlormequat and related pesticides.

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A review of the literature shows that currently there are no methods available for the simultaneous measurement of chlormequat (CQ), difenzoquat (DFQ), diquat (DQ) and paraquat (PQ) in waters or any other matrix. A variety of methods have been published for these individual compounds, although most publications have been concerned with mixtures of diquat and paraquat. This is probably due to the wide usage of these compounds for many years. The 1987 'MEWAM' method for paraquat and diquat employed reduction with alkaline sodium dithionite and determination of the reduced compounds by visible light spectroscopy or 2nd derivative measurement. Although this method is sensitive it requires up to 5L water samples, and cannot be used when both herbicides are present in the same sample. Gill *et al* [1] showed in 1983 that HPLC with UV detection could detect PQ and DQ to about 1µg/ml urine in six minutes run time. Many authors have used this HPLC system for a wide range of matrices, and the method is probably very robust. The sensitivity obtained by Gill is equivalent to 1mg/litre, which would require a sample concentration factor of 10,000 to be incorporated into any new method to achieve a target detection level of 0.1µg/litre. Although it should be possible to improve the sensitivity of the Gill method by using 2mm i.d columns, and that the same method should be suitable for difenzoquat, it would not detect chlormequat. This is because chlormequat is almost undetectable by UV. This is the major problem facing the analysis of this compound.

There are few publications for chlormequat that could be considered suitable to replace the current MEWAM method, which requires a reagent that is no longer available. In 1970, Tafuri *et al* [2] showed that derivatisation of chlormequat with sodium benzenethiolate yielded a product that could be analysed by gas chromatography. This approach has been improved by Allender [3] in 1992 who used the related compound pentafluorothiophenol to yield a product that is highly detectable to GC-electron capture systems. However, the product is rather too volatile and elutes close to the solvent front, and the reaction produces other by-products that require the GC to be temperature programmed over a period of 35 minutes. It is not clear what effect this reaction would have upon diquat, paraquat and difenzoquat, but it is possible that after the initial de-quaternisation step the reaction may stop. If that were the

case, then it is possible that the products could be chromatographed by GC, although the conditions would require much higher temperatures than the chlormequat product, and detection may be complicated by the large number of unidentified peaks arising from the chemical derivatisation. The 'MEWAM' method for difenzoquat that utilises GC-NPD following conversion of DFQ to the monomethyl pyrazole derivative in the heated injector, is unlikely to be suitable for chlormequat, diquat and paraquat. It is , in any case, a method that depends upon the performance and suitablility of a particular instrument, and is unlikely to produce a robust method. In general, quaternary ammonium compounds are considered unsuitable for analysis under GC conditions, and in this consultancy were given a low priority. No new data for the use of the pentafluorothiophenol reagent was generated.

The fact that chlormequat, difenzoquat, diquat and paraquat are all quaternary ammonium compounds means that it should be possible to exploit this common chemical property in order to develop a single analytical method for their measurement. This consultancy has investigated the HPLC method [4] that uses post-column ion-pair formation between organic amines with 9,10-dimethoxyanthracenesulphonic acid coupled with on-line extraction into an immiscible organic solvent followed by fluorescence detection. The equipment contains an aqueous-organic phase separator designed by Jefferies that is responsible for supplying a clean, droplet-free flow of organic extract to the detector [5]. This complete post-column unit is now commercially available, and can be used with any HPLC system. Although post-column HPLC is more complex than simple HPLC, the equipment is robust in operation and suitable for routine use.

Results

Although the four compounds are quaternary ammonium compounds there are major differences in chemical structure between them. Chlormequat is a very small, aliphatic hydrophilic compound without any UV absorbance; diquat and paraquat are both aromatic compounds with good UV absorbance and two quaternary nitrogens; difenzoquat is also aromatic with good UV absorbance and but has only one quaternary nitrogen. All four compounds are able to form ion-pairs with acidic compounds such as 9,10-dimethoxyanthracenesulphonic acid, sodium salt, but the extraction of these complexes into an organic solvent such as dichloroethane depends upon the hydrophobicity of the complex.

It has been found that diquat and paraquat are only poorly detected even at the 1 $mgml^{-1}$ level, which can be explained by proposing that only one of the two quaternary nitrogens

is complexed due to steric hinderance, and that the free quaternary nitrogen makes the complex suffiently polar to keep it water soluble. Chlormequat is detectable at the 1 μgml^{-1} level, which is not as sensitive as expected because the alkyl chain is only two carbon atoms long which means that the properties of the chlorine atom are influenced by the adjacent quaternary nitrogen, i.e. it is more polar. Choline chloride is poorly detected because the hydroxyl group is highly polar which keeps the ion-pair complex soluble in water. It can be detected above about 100 μgml^{-1} . Difenzoquat is detectable at the 0.1 μgml^{-1} level because when the quaternary nitrogen is complexed, the remainder of the molecule is relatively hydrophobic.

The work to date concentrated on finding suitable chromatographic conditions to retain such a highly water-soluble compound as chlormequat, without using an ion-pair reagent in the mobile phase because this would compete with the ion-pair reagent in the post-column system. Polymeric strong cationic exchange columns were exhaustively tested from Polymer laboratories and Hamilton Inc., but lacked column efficiency under the weak solvent conditions required for the post-column system. Polymer Laboratories even made a special material to improve column efficiency. This arrived two weeks ago and has not yet been tested. However, a silica based weak cation exchanger from Whatman (Partisphere-WCX) provided better peak shapes and less retention than the polymeric columns, and so work has concentrated on this column for the past month. The post-column system has been designed to achieve maximum sensitivity in all applications by using narrow (2 mm id) columns which should produce peaks 3 to 4 times taller than those from a standard (4.6 mm id) column. Narrow columns also require lower flowrates (0.3 $mlmin^{-1}$) than standard columns (1 $mlmin^{-1}$) and so are cheaper to run. The report describes work carried out with a standard column at a compromise flowrate of 0.6 $mlmin^{-1}$ which makes the peaks more broad than they could be and reduces sensitivity. Whatman cannot supply a 2 mm Partisphere-WCX column but are prepared to supply packing material to Capital HPLC Ltd in order to pack a column for this application. A column is expected shortly.

The chromatographic conditions have been "tuned" to reduce retention of chlormequat from about 16 minutes to about 10 minutes in order to produce taller peaks and so improve sensitivity. Choline chloride now has a retention time of 8 minutes and difenzoquat 26 minutes. Conditions could be ghanged to reduce the retention of difenzoquat, but chlormequat and choline chloride would not be completely separated.Factors influencing baseline noise were also studied in order to reduce it to a minimum so that the amplification of the detector could be increased. It has

been found that baseline stability is considerably improved by equilibration of the column, without the post-column unit, overnight at 0.2 $mlmin^{-1}$ with the mobile phase. The complete system is ready for use within 15 minutes of connecting to the post-column unit next day, and remains stable throughout the day. No other cleaning washes for the column have been found to be needed to date, although experience with hard water samples is limited.

The development of a recovery procedure for chlormequat from distilled water and from tap water has only recently begun because until an HPLC system was available, recovery measurements could not be made. Solid phase extraction cartridges are the method of choice because they provide reliable procedures that can be carried out in batches of samples. Using knowledge gained from working with the Partisphere-WCX column, a similar material has been studied in a cartridge and has provided about 60% recoveries from distilled water and 25% recoveries of 10 µg chlormequat from 100 ml tap water (containing about 100 ppm Calcium and about 20 ppm magnesium).

Attempts to improve these recoveries by adding the chelating agent EDTA to the tap water has not been successful. It also causes a large unknown peak to be eluted at about 8 minutes, such that chlormequat is eluted on the falling shoulder of this peak. Another approach that could be considered for any future study would be to examine the performance of larger CBA cartridges, such as the 3g. It may also be useful to examine the current 'MEWAM' method that evaporates a 1L sample down to 10 ml, and then attempt to remove calcium and magnesium at that stage, before trapping chlormequat on the CBA cartridge. No studies have been carried out on thge recovery of difenzoquat, but it is expected to be recovered at higher rates than chlormequat, under similar conditions because it is more hydrophobic and therefore less easily removed from the cartridge.

The lowest levels that can be achieved for chlormequat and difenzoquat determinations in waters depends upon the % recoveries from waters and the volumes of samples that can be conveniently used with cartridges. The manufacturer of the cartridges being tested recommends a flow rate of 2 $mlmin^{-1}$ for maximum recovery values, ie a 500 ml sample would require 4 hours to be passed through the cartridge. This is not a restriction in its use because this step should require little attention, however it is a factor in considering sample volumes. Currently, this study has demonstrated that HPLC with post-column extraction and fluorescence detection can form the basis for a method to determine chlormequat and difenzoquat, but not diquat or paraquat, in the presence of choline chloride. Sensitivities in the desired range of 0.1 μgL^{-1} are not

achievable. If the recovery of chlormequat can be increased to 50% from a 500 ml sample, then assuming the HPLC sensitivity remains at 1 μgml^{-1} for chlormequat and 0.1 μgml^{-1} for difenzoquat, then the limits of detection would become $4\mu gL^{-1}$ and 0.4 μgL^{-1} respectively.

Capillary electrophoresis

This analytical technique is rapidly becoming an important additional, and complimentary procedure to HPLC and GC. It is ideally suited to ionisable compounds, and can resolve compounds of very similar chemical structure. The columns are as efficient as those used in GC, and running costs are less than either HPLC or GC. We have found that it is possible to separate DFQ, DQ and PQ in 10 minutes. The detection was by UV and so chlormequat was not included in this preliminary study. However, it is possible to detect non-UV absorbing compounds such as chlormequat by the addition of a UV-absorbing amine in the running buffer solution, so that the chlormequat is detected as a negative peak. The sensitivity depends upon the sensitivity of the added amine, and a report from one manufacturer of capillary electrophoresis equipment has claimed at least 1mg/litre detection for choline chloride using this method, electropherogram enclosed. This approach has not been investigated any further due to the lack of equipment. Cai *et al* have published a CE method [6] for diquat and paraquat that resolves these compounds and an internal standard in 12 minutes. Detection on this in-house made system was by UV and sensitivity was down to 0.4µg/ml, (0.4mg/litre). This could be improved upon considerably using commercial equipment. A CE instrument will shortly be available to DR Jefferies and so this technique could be examined for its capabilities for chlormquat and related herbicides.

References

[1] Gill, R, Qua, S.C. and Moffat A.C., *J.Chromatogr.*, 1983, **255**, 483-490.
[2] Tafuri, F., Businelli, M and Giusquiani, P.L., *Analyst*, 1970 **95**, 675-679.
[3] Allender, W.J., *Pestic.Sci.* 1992, **35**, 265-269.
[4] Roy, I.M., Jefferies, T.M., Threadgill, M.D and Dewar, G.H., *J.Pharm.& Biomed.Anal.* 1993, **10**, 943-948.
[5] Roy, I.M. and Jefferies, T.M., *J.Pharm.& Biomed. Anal.*, 1990, **8**, 831-835.
[6] Cai, J. and el Rassi, Z., *J.Liq.Chromatogr.*, 1992,**15**,1193-1200.

Chlormequat in Waters.

Tentative method 1994

Methods for the Examination of Waters and Associated Materials

Chlormequat in waters by high performance liquid chromatography with post-column ion-pair formation and on-line extraction.
Tentative Method 1994

1 Performance characteristics of the method

1.1 Substances determined	Chlormequat, (chlorocholine chloride, 2-chloroethyl-trimethylammonium chloride)
1.2 Types of sample	Drinking water.
1.3 Basis of method	The samples are extracted using a solid phase extraction cartridge, the isolated extract is evaporated to dryness, the residue dissolved in the HPLC mobile phase, and the chlormequat determined by post-column HPLC with fluorescence detection.
1.4 Range of application	To be determined.
1.5 Calibration curve	The method is linear over the range of application. Using standard solutions of chlormequat, peak heights were linear over the range of 1 to 10 $\mu g ml^{-1}$
1.6 Standard deviation	To be determined
1.7 Limit of detection	To be determined.
1.8 Sensitivity	Dependent on the instruments used.
1.9 Bias	Extraction efficiences are normally less than 100%.
1.11 Time required for analysis	Approximately x samples per day.

2 Principle

Chlormequat is extracted from water using an ion-exchange solid phase extraction cartridge (SPE), removed from the cartridge using acidified acetonitrile, and concentrated to incipient dryness by evaporation. The residue is

dissolved in water and analysed by ion-exchange HPLC with post-column ion-pair formation with DAS and on-line extraction using dichloroethane. Detection is by fluorescence.

3 Interferences

Any material that has a similar retention time on the HPLC column that is also capable of forming an ion-pair with 9,10-dimethoxyanthracenesulphonic acid (DAS) which is then extracted into dichloroethane. Some organic amines are potentially able to interfere, although under the HPLC conditions selected here, most compounds would be more retained than chlormequat. Choline (2-hydroxyethyltrimethylammonium chloride) is recovered by the SPE cartridge and is resolved from chormequat by the HPLC system. However, it is even more polar than chlormequat, so that although it forms an ion-pair with DAS, this complex is mainly soluble in water and is only very weakly extracted into dichloroethane. It is therefore detected only above about 100 $\mu g ml^{-1}$.

4 Hazards

Due to the hazardous nature of solvents such as dichloroethane and acetonitrile, all preparation should be carried out in a fume cupboard. Ensure adequate ventilation and work in a flame and spark free area. Inhalation may cause dizziness, headache, nausea and mental confusion. Vapour irritating to eyes and mucous membranes. Harmful by ingestion and skin contact. Wear safety spectacles and appropriate protective clothing. Chlormequat is toxic.

5 Reagents

All reagents must be of sufficient purity that they do not give rise to significant interfering peaks during the

analysis. This should be checked for each batch of material and verified by running procedural blanks with each batch of samples analysed.

The water used for blank determination and preparation of control samples should have negligible interferences in comparison with the smallest concentration to be determined.

Reagents may become contaminated by contact with air and other materials,particularly plastics, or by degradation caused by the action of light. Reagents should be stored in the dark in tightly sealed all-glass containers or other vessels found to be suitable.

5.1	**Acetonitrile**	HPLC grade
5.2	**Dichloroethane**	HPLC grade
5.3	**Methanol**	HPLC grade
5.4	**Water for HPLC.**	Ultrapure.
5.5	**HPLC mobile phase**	Acetonitrile - 0.015M potassium dihydrogen phosphate (pH 5.5, by the dropwise addition of KOH solution) (22:78 v/v).
5.6	**Reagent solution**	12.5 mgL^{-1} 9,10-dimethoxy-anthracenesulphonic acid sodium salt
5.7	**Organic phase**	dichloroethane
5.8	**Standard solutions:**	anthracenesulphonic acid sodium salt in ultrapure water.
5.8.1	**Stock solutions.**	10 mg chlormequat in 10 ml HPLC grade water. 10 mg choline in 10 ml HPLC water. 10 mg difenzoquat in 10 ml HPLC grade water.
5.8.2	**Working solutions**	10 µg chlormequat in 100 ml water

5.9 Nitrogen. Oxygen free, filtered and dry.

6 Apparatus Apparatus should be free from contamination before use. Glassware should be rinsed immediately before use with methanol and then dichloromethane and allowed to drain.

6.1 Glass sample bottles 1 L capacity, with PTFE lined screw caps.

6.2 Solid phase extraction

6.2.1 Cartridge system A vacuum unit is required that can accommodate about ten cartridges. Control of the flow-rate of sample through the cartridges is important for reproducible results, and is best achieved by a tap for each cartridge. All components of the unit should be either glass or PTFE. Steel components may be a cause of interferences in the detector. A suitable unit may be obtained from Jones Chromatography Ltd, (Hengoed, Mid-Glamorgan).

6.2.2 Cartridge 500 mg weak cation exchanger, silica based with carboxylic acid functionality, such as Isolute-CBA cartridge (Jones Chromatography), 10 ml volume, fitted with adaptor and 75 ml reservoir.

6.3 HPLC system with post-column unit and fluorescence detector

6.3.1 HPLC system An isocratic system is required capable of providing a pulse-free flow at 0.6 $mlmin^{-1}$. The column required is a weak cation exchanger, 125 x 4.6 mm id. Partisphere WCX (Whatman) is a suitable material, but similar materials from other manufacturers may also be satisfactory. The injection valve was fitted with a 20µl loop.

6.3.2 Post-column unit

The unit tested was manufactured by Scientific Systems Inc., (State College, PA, USA), and is available in the U.K. from Spectronic Services Ltd, (Garforth, Leeds). The performance of units available from other manufacturers would need to be examined for their suitability for this method. The configuration of the HPLC post-column system is shown in Figure 1, and its performance described in reference [10.1]. The aqueous reagent pump and organic phase pump are model 350 pumps fitted with Lo-pulse dampeners. Additional pulse dampening may be provided by two 100 x 4.6 mm id columns containing 10 μm silica. The addition of the aqueous reagent to the HPLC eluent and the segmentation of the aqueous phase was achieved using Tee-connectors, 1/16 x 0.015 inch id. The extraction coil is 1.5 m x 0.8 mm id s/s tubing. The phase separator and its performance have been described in reference [10.2]. It is designed to deliver a clean, water-free, flow of organic phase to the detector, and its output is controlled by two MCV-50 microneedle valves (Scientific Glass Engineering Ltd, Milton Keynes).

7 Sample preservation and storage

Samples should be extracted and analysed as soon as possible after sampling. If this is impractical, they should be stored in a refrigerator at about 4 ^{0}C. The sample bottles should be protected from contamination and should not be placed in the close proximity of standard materials or their concentrated solutions.

8 Analytical procedure

Step	Procedure	Notes
8.1	**Solid Phase Extraction**	
	Flow rate to be approximately 2 ml min^{-1}	
	The following solutions to be used in sequence:	
(a)	5 ml methanol	(a) This step conditions the cartridge for use. Do not permit the cartridge to dry out at any stage.
(b)	10 ml 0.05M KH_2PO_4 pH 6.5 5 ml 0.01M KH_2PO_4 pH 6.5	(b) This step ensures the ionisation of the CBA functionality and then leaves the cartridge in the same strength buffer as the water sample.
(c)	Water Sample, 100 ml, plus 1 ml 1M KH_2PO_4 pH 6.5. Mix thoroughly.	(c)Drinking water may develop a white precipitate due Ca^{2+} Filter into the cartridge reservoir using a Whatman No 1 filter paper. Monitor flowrate.
(d)	5 ml Acetonitrile containing 0.1% trifloroacetic acid.	(d) CBA ionisation is suppressed, releasing chlormequat. Collect. in test-tube.
(e)	5 ml Acetonitrile containing 0.1% trifloroacetic acid.	(e) Second step ensures all chlormequat is collected.
	Evaporate the two chlormequat extracts to incipient dryness using nitrogen plus heat at 40°C.	Store at 4°C
8.1.2	**HPLC analysis**	
	Set up the HPLC in accordance with the manufacturer's instructions. The column should be conditioned overnight with the mobile phase at 0.2 ml min^{-1} before use.	

Switch off the HPLC pump and connect to the post-column unit as Figure 1. Switch on the organic phase pump and ensure that clean dichloroethane is flowing through the detector. Switch on the detector. Switch on the HPLC pump at 0.6 ml min^{-1} and check that clean organic phase is flowing through the detector. Switch on the reagent pump and allow about 15 minutes for the baseline to settle before use. Adjust the flowrate of organic phase through the detector using the microneedle valves to about 0.5 ml min^{-1}.

8.1.3 Inject aliquots of standard solutions into the HPLC to establish the retention time of chlormequat (10 mins), choline (8 mins) and difenzoquat (26 mins) and the sensitivity achieved.

8.4 Blanks.

8.4.1 Adequate blank values should be obtained using interference free water before analysing samples.

8.5 Standards.

8.5.1 Inject a chlormequat standard solution to determine the performance and sensitivity of the system, and use as an external standard for samples.

8.6 Samples

8.6.1 Add 500 µl ultrapure water to the dry residue in each test-tube from 8.1 step (e), vortex mix for 15 seconds to dissolve residue, take up into a 1 ml Luer syringe, remove the needle and replace with a small membrane filter (0.45 µm) such as 13 mm, suitable for aqueous solutions, and filter into a suitable vial. Inject 20 µl into the HPLC, in duplicate.

8.7 Recoveries

Results to date indicate a 60% recovery of 10 µg in 100 ml ultrapure water and 25% recovery from tap water containing about 100 ppm Ca^{2+} ions and 20 ppm Mg^{2+} ions.

9 Calculations

Compounds are under examination for their suitability as a recovery standard, which would improve reproducibility.

10 References

10.1 I.M.Roy, T.M.Jefferies, M.D.Threadgill and G.H.Dewar, "Analysis of cocaine, benzoylecgonine, ecgonine methyl ester, ethylcociane and norcocaine in human urine using HPLC with post-column ion-pair extraction and fluorescence detection", J.Pharm. Biomed Anal. **10**, (1993) 943 - 948.

10.2 I.M.Roy and T.M.Jefferies, "Performance evaluation of an Aqueous-organic phase separator for post-column reactions in high performance liquid chromatography, and its application to the enhanced detection of some drugs of abuse", J.Pharm Biomed. Anal, **8**, (1990), 831 - 835.

27 February 1994

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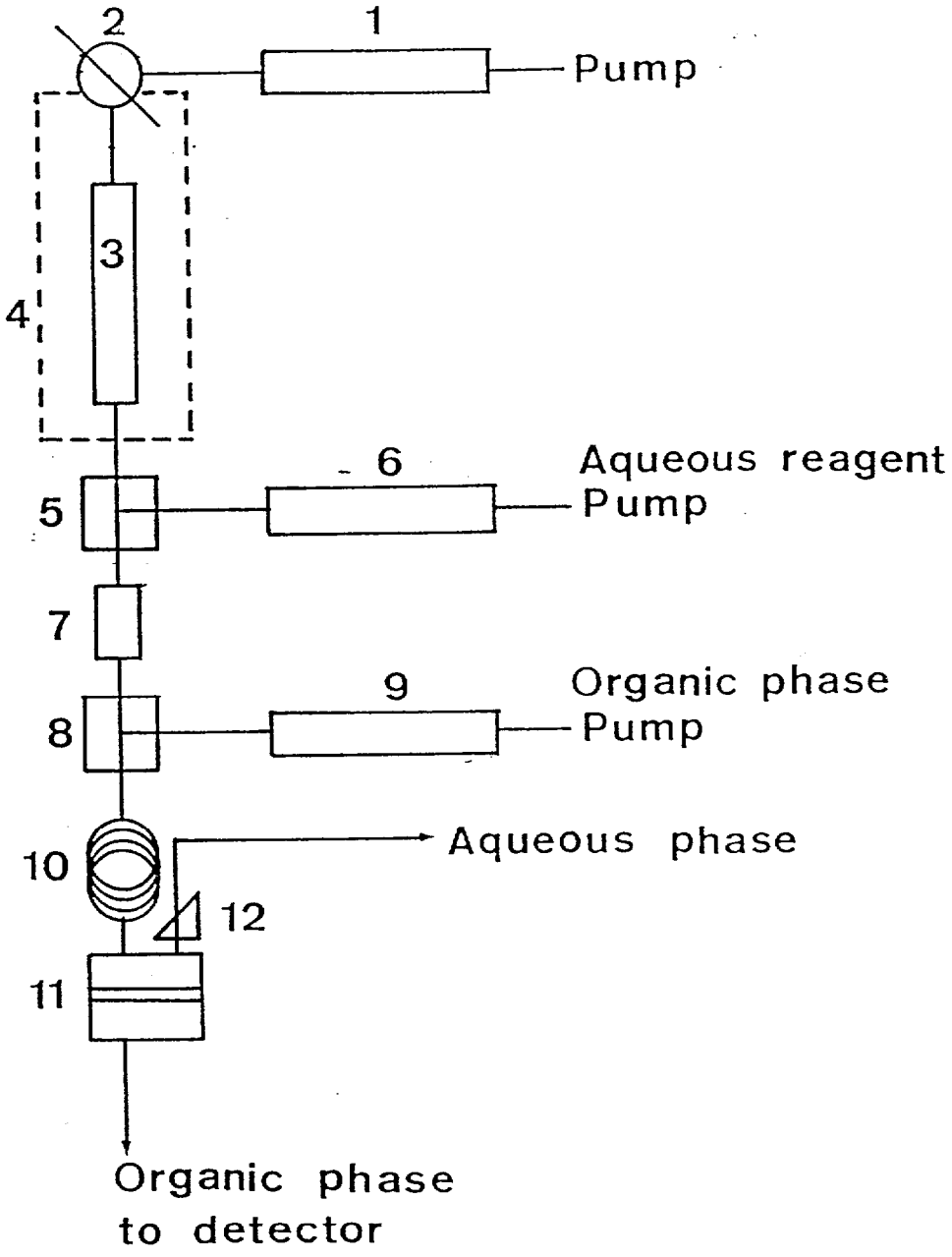
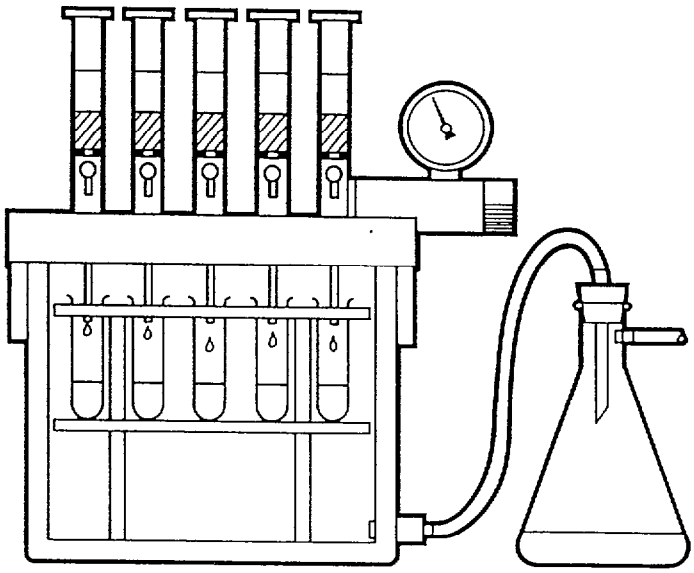


Figure 1

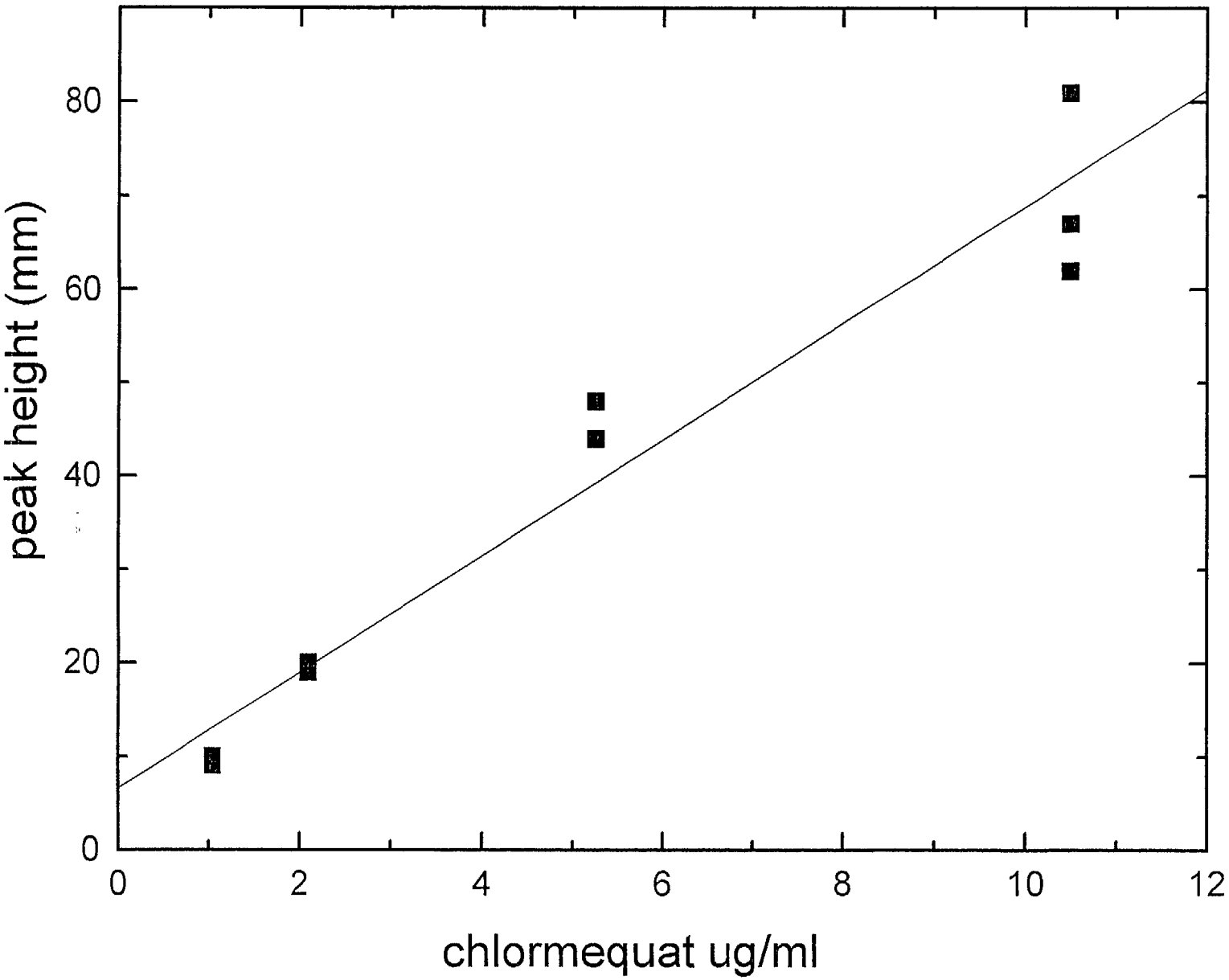
Schematic diagram of HPLC post-column system.
1 = silica 10 µm particle column to protect analytical column from mobile phase; 2 = injection valve; 3 = analytical column; 4 = optional column oven, may be used at 30°C; 5 and 8 = Tee connectors; 7 = mixing column or mixing connector; 6 and 9 = optional 10 cm silica 10µm particle columns to provide additional pulse dampening if required; 10 - extraction coil; 11 = phase separator; and 12 = microneedle valves to control organic flow to detector.

Figure 2

Vacuum manifold for Solid phase extraction unit.



Vacuum Manifold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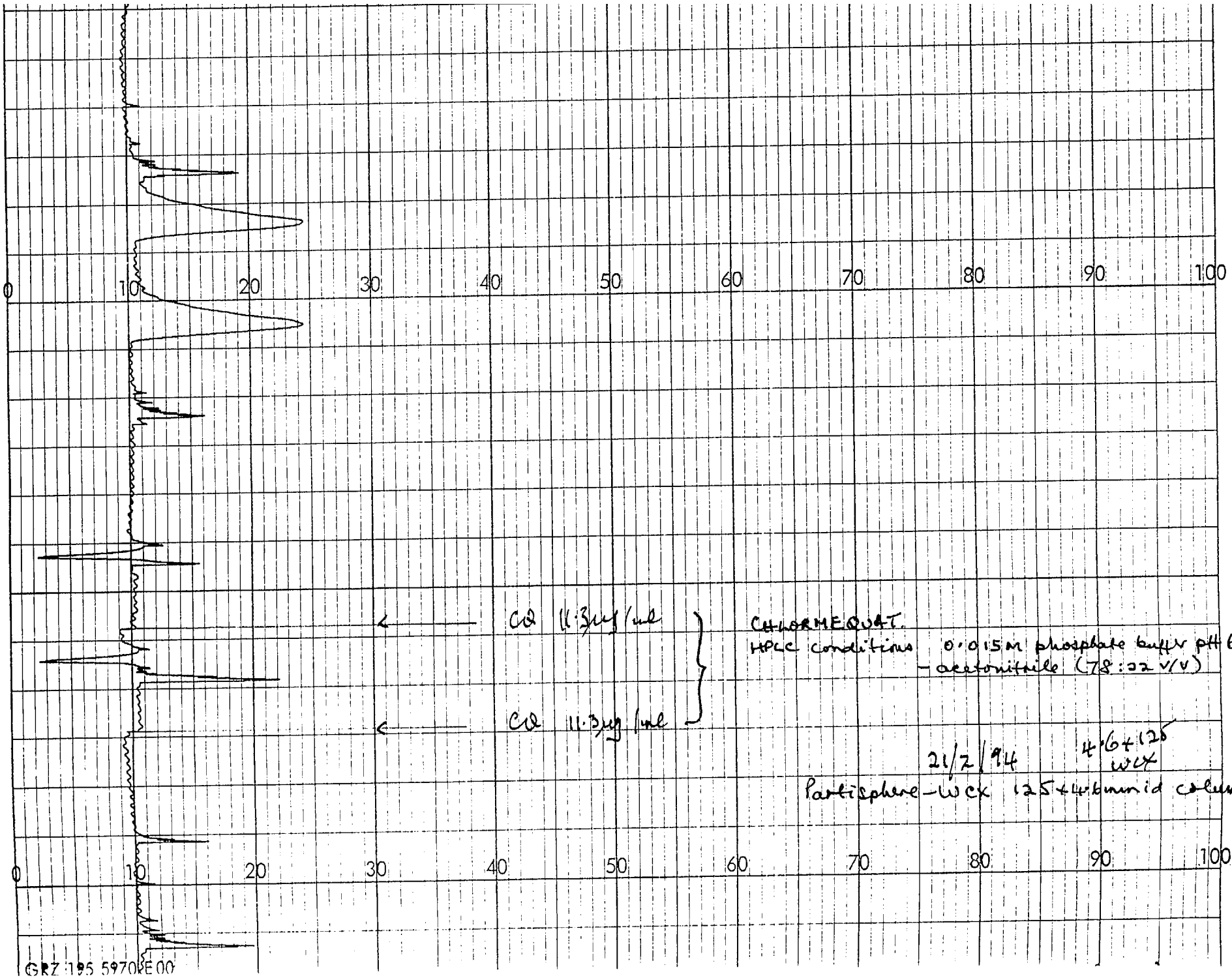
20
40
60
80
0
2
4
6
8
10
12
chlormequat ug/ml


0
10
20
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CQ 11.3µg/ml
CQ 11.3µg/ml
CHLORMEQUAT.
HPLC conditions 0.015M phosphate buffer pH 6.0
– acetonitrile (78:22 v/v)
21/7/94
4.6+125 WCX
Partisphere-WCX 125+4.6mm id column.
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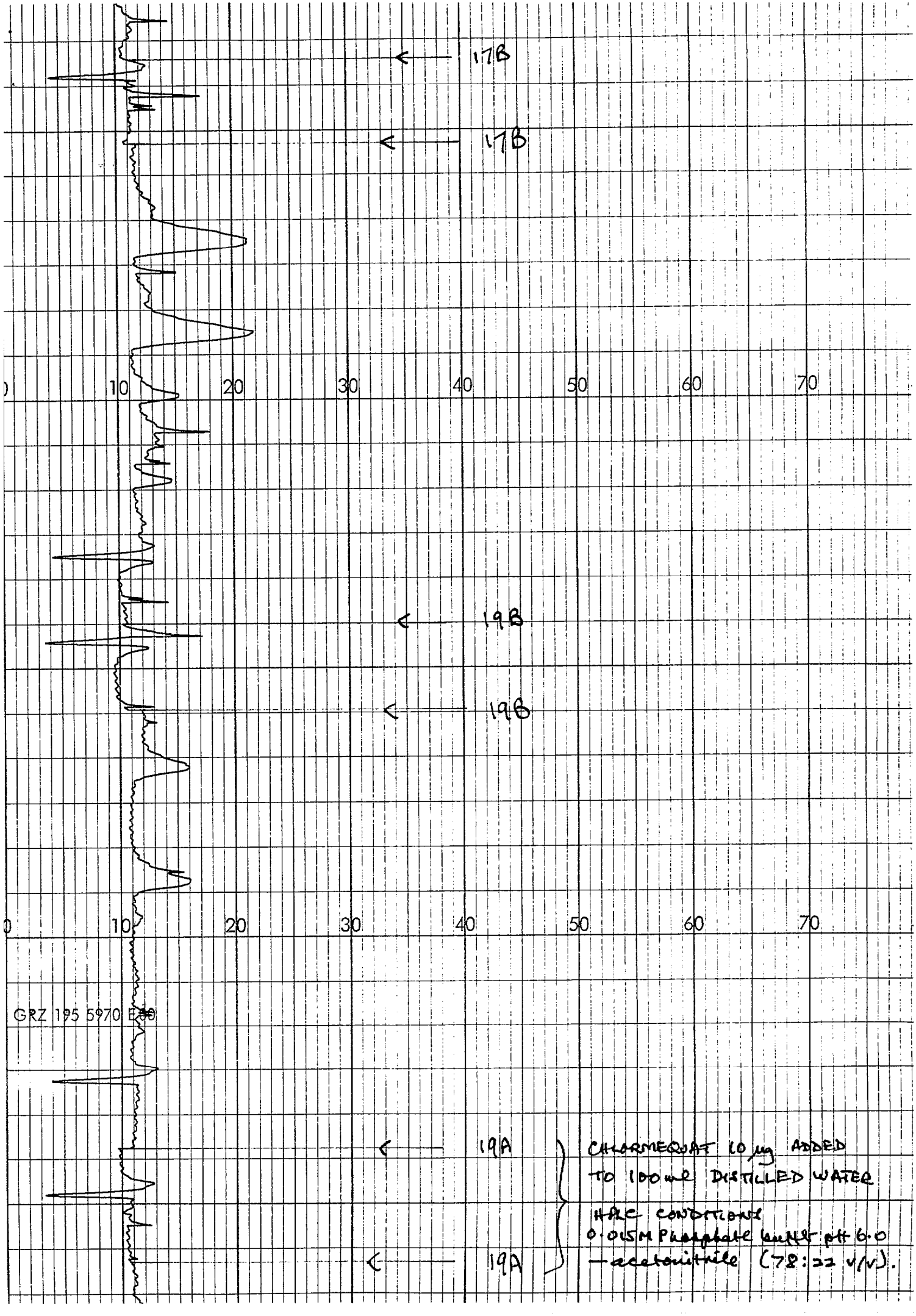
GRZ 195 5970kE 00


17B
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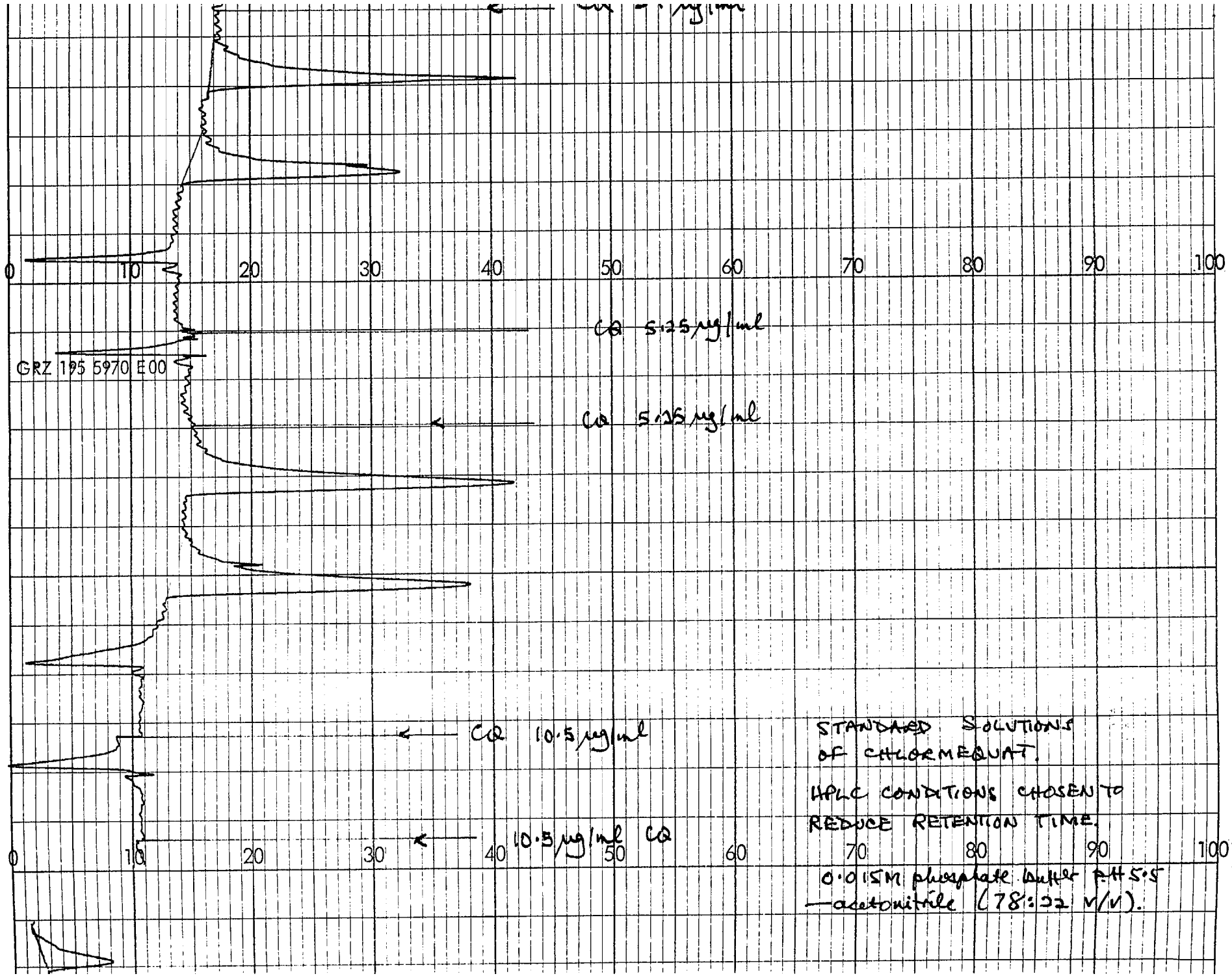
19A
19A
CHLORMEQUAT 10 µg ADDED
TO 100 ml DISTILLED WATER
HPLC CONDITIONS:
0.045M Phosphate buffer pH 6.0
— acetonitrile (78:22 v/v).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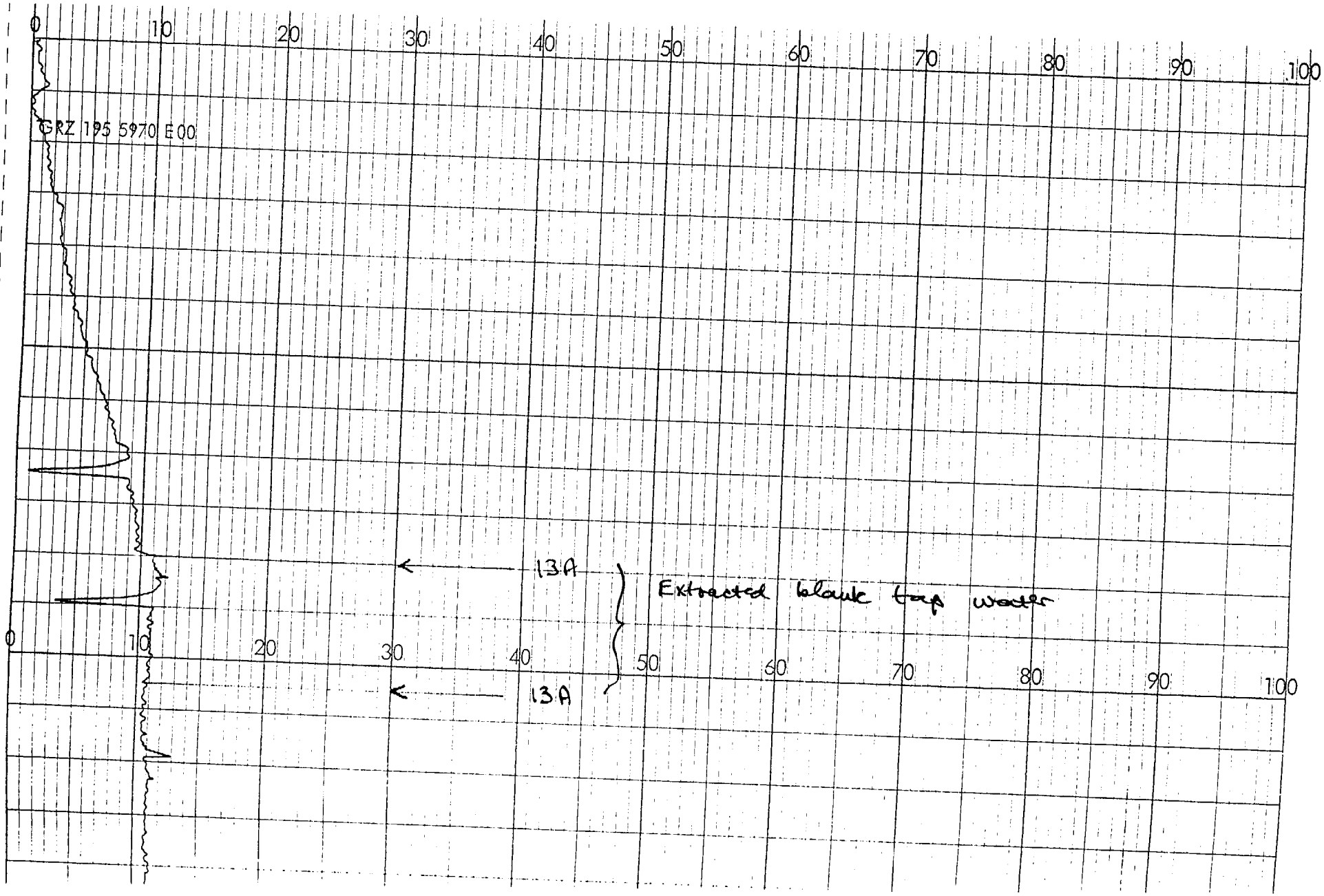
CQ 5.25 µg/ml
GRZ 195 5970 E00
CQ 5.25 µg/ml
CQ 10.5 µg/ml
10.5 µg/ml CQ
STANDARD SOLUTIONS OF CHLORMEQUAT.
HPLC CONDITIONS CHOSEN TO REDUCE RETENTION TIME.
0.015M phosphate buffer pH 5.5 — acetonitrile (78:22 v/v).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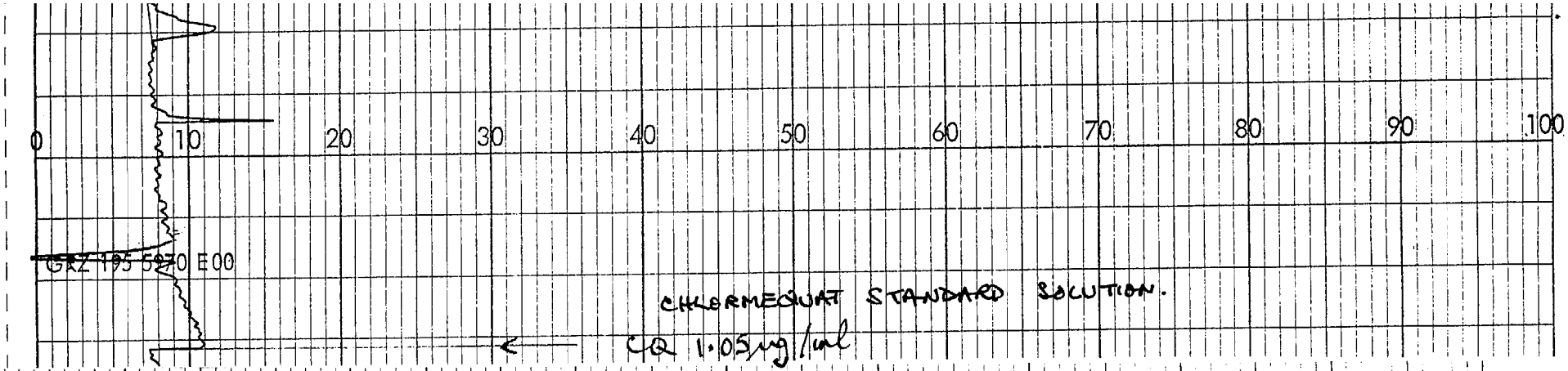
GRZ 195 5970 E00
13A
Extracted blank tap water
13A


0
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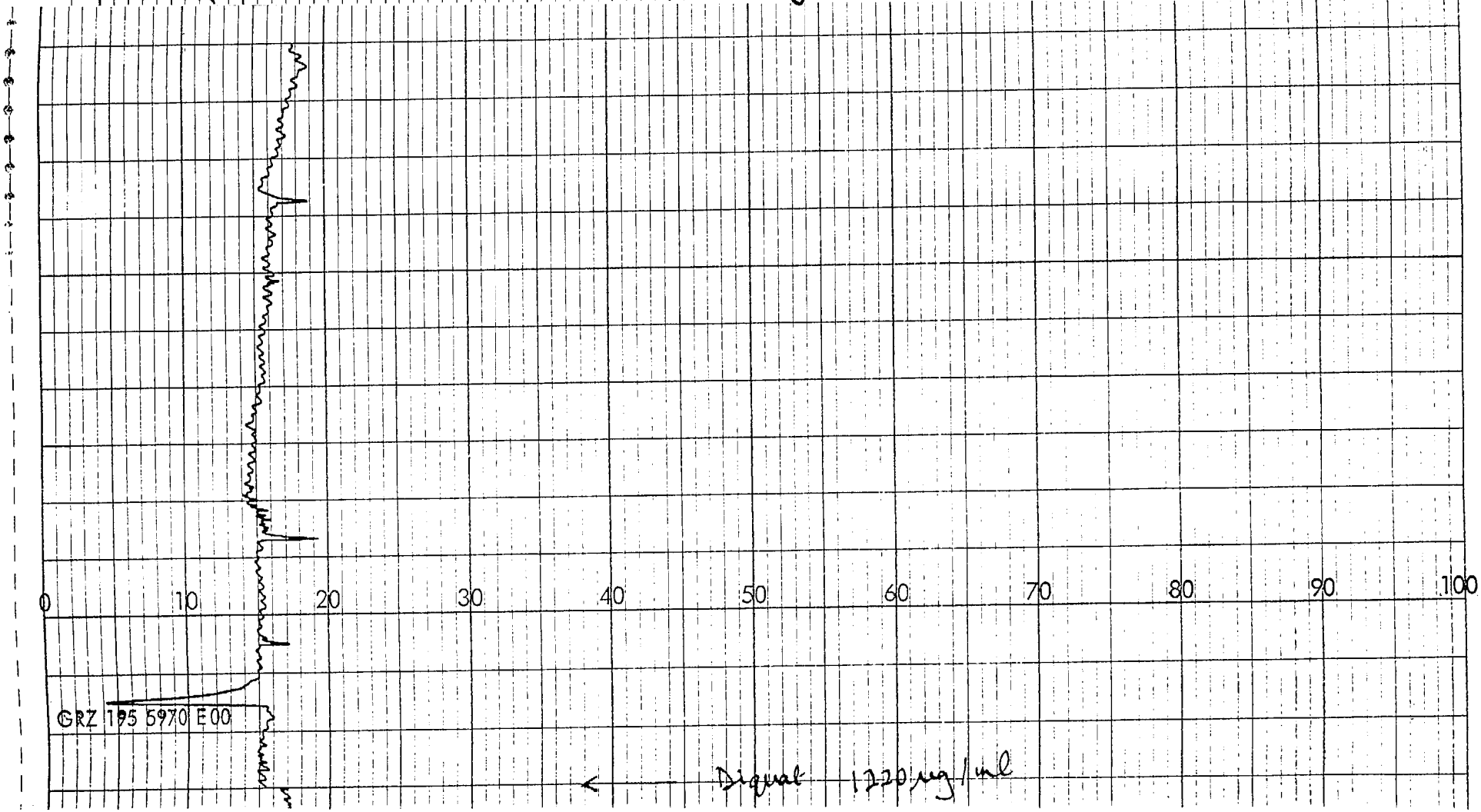
GRZ 195 5970 E00
CHLORMEQUAT STANDARD SOLUTION.
CQ 1.05 mg/ml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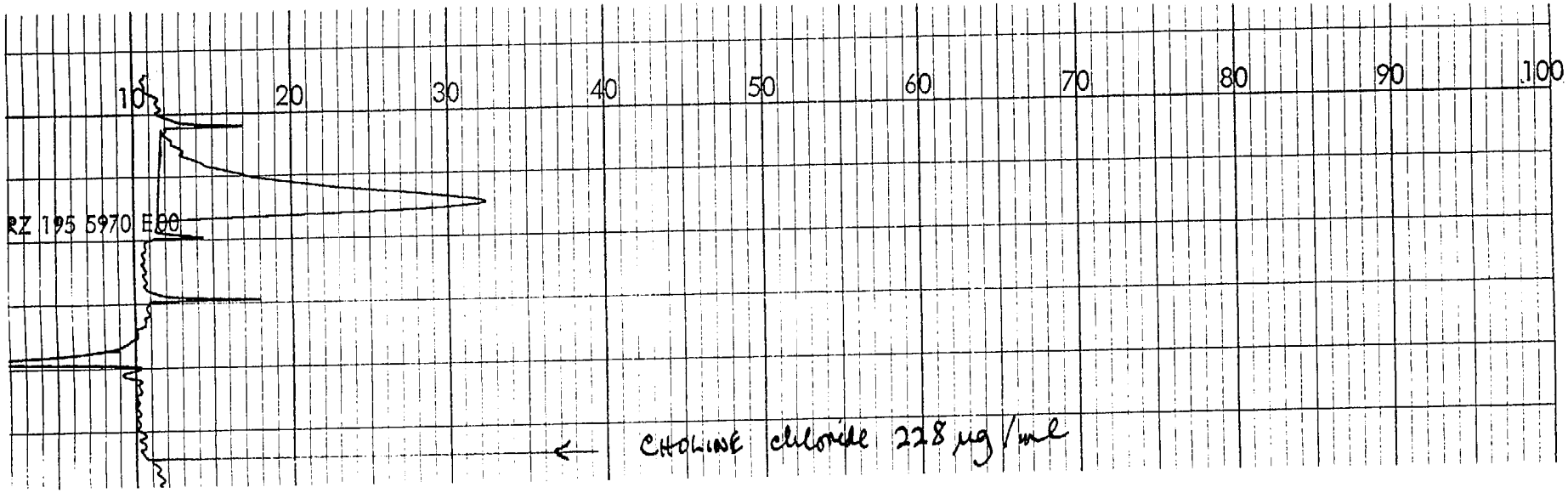
100
GRZ 195 5970 E00
Diquat 1220 mg/ml


10
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80
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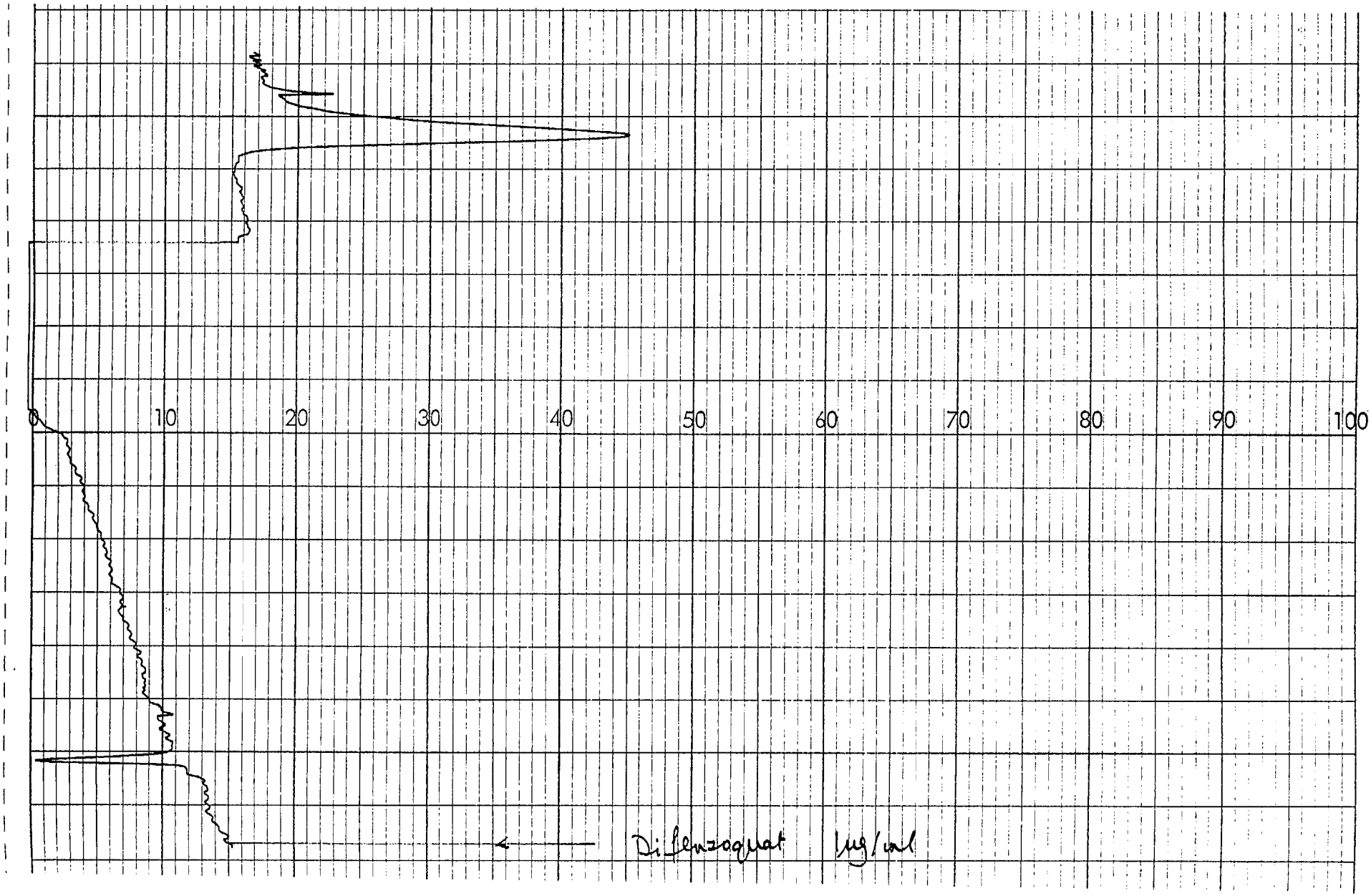
100
RZ 195 5970 E00
CHOLINE chloride 228 µg/ml


0
10
20
30
40
50
60
70
80
90
100
Dibenzoquat µg/ml

Data Reprocessed On 11/17/1992 17:46:31

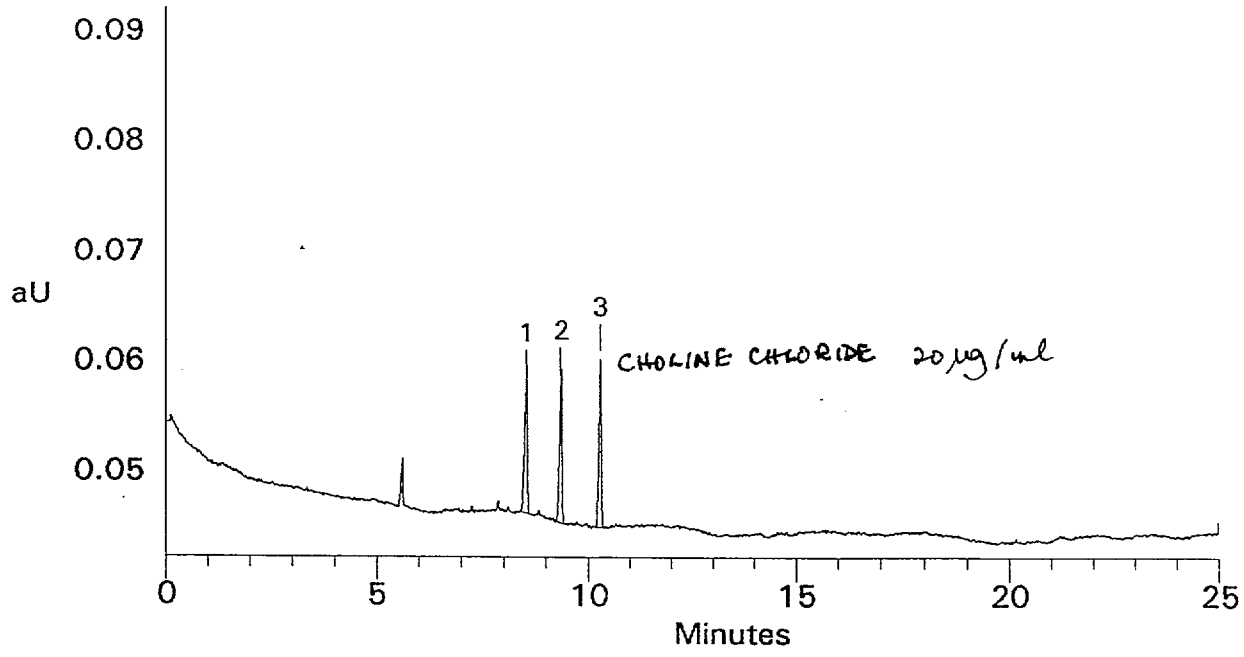
```
ample Name:                                    Date: 10/22/1992 15:31:04
ata File   : C:\DX\DATA\CES\PH000031.D05
ethod      : C:\DX\METHOD\CES.MET
CI Address: 1  System: 2  Inject#: 5                    Detector:UV/Vis
nalyst     :                Column:
```

ibration	Volume	Dilution	Points	Rate	Start	Stop	Area Reject
ernal	1	1	7500	5Hz	0.00	25.00	0

********** Peak Report: All Peaks **********

:. m	Ret Time	Component Name	Concentration PPM	Height	Area	Bl. Code	%Delta
1	8.53	Trimethylamin HCL	20.000	22753	118403	1	0.04
2	9.35	TMVAH	20.000	24984	116369	1	0.00
3	10.28	Choline Chloride	20.000	30019	118101	1	0.03
		Totals	60.000	77756	352873		

File: PH000031.D05 Sample:



CAPILLARY ELECTROPHORESIS.

USING INDIRECT UV TO DETECT CHOLINE CHLORIDE